many genes may be processed in parallel. Considering that every gene is a potential mutagenesis target, the proposed approach facilitates the generation of extensive libraries of mutated mammalian genes, as well as libraries of pluripotent stem cells carrying those gene mutations.

Other features and advantages of the invention will be apparent from the following detailed description and from the claims.

Detailed Description

The drawings will first briefly be described.

FIGURE 1 is a schematic representation of a Moloney murine leukemia virus (MoMLV)-based vector for use in the MAGEKO process.

FIGURE 2 is a schematic representation of an insertional mutagenesis event.

FIGURE 3 is a schematic representation of the MAGEKO process of insertional mutagenesis in an exon sequence.

FIGURE 4 is a schematic representation of the MAGEKO process of insertional mutagenesis in an intron sequence.

The present invention involves vectors and a process, termed "MAGEKO" (or "massively parallel gene knock out") which permits the mutagenesis of large numbers of mammalian genes, the creation of libraries containing those mutant genes, and the ready selection from that library of stem cells carrying mutant genes of interest. Although this process is applicable to any mammalian system, it is now described for the generation of mutations and libraries in a mouse system. The following examples are presented for the purpose of illustrating the invention, and should not be construed as limiting.

The MAGEKO Process

The MAGEKO process involves retroviral insertional mutagenesis, on average every 1 Kb in the mouse genome, to create a comprehensive library of KO ("LOK") embryonic stem (ES) cells, and a gene KO identification system ("KIS"). The LOK generally includes mutations in every mouse gene, and the KIS allows the rapid isolation of desired mutant ES cells. The LOK and KIS facilitate the large scale automated search for KO cells potentially corresponding to any desired gene.

Once appropriate ES cells are identified, ES cell-derived embryos are generated in vitro, by aggregation with tetraploid or morulae stage embryos (for example, by the method of Wood et al., Nature **365**:87-89 (1993)). These embryos are subsequently implanted into foster mothers for the generation of heterozygotic mice with a KO in the gene of interest. Conventional blastocyst injection methods can also be employed, if appropriate (see, for example, Robertson, Trends Genet. **2**:9-13 (1986)). Heterozygotic mice are converted to homozygotes through mating.

In parallel, the heterozygotic mutant ES cells may also be converted to homozygotic cells in vitro, according to published protocols (for example, Mortensen et al., Mol. Cell. Biol. **12**:2391-2395 (1992)), and used to generate homozygotic mice with the above described techniques. The homozygotic mice obtained by either method may be analyzed to determine the function of the knocked out gene of interest.

The MAGEKO Components

The MAGEKO process broadly encompasses three components: (i) the generation of gene mutations in mammalian genes using retroviral vectors; (ii) the production of libraries of knocked out genes which may be used to generate mutant animals; and (iii) the selection of cells carrying mutations in desired genes. Each of these components is now discussed.

(I) Components of the Retroviral Vectors

Retroviruses are RNA viruses which replicate through a DNA intermediate and which include as an obligatory step of their life cycle integration of the proviral DNA into the host chromosome (Varmus and Brown, Retroviruses. In *Mobile DNA* (ed. Berg, D. E. and M. M. Howe), pp. 53-108. American Society for Microbiology, Washington, D.C. (1989)). Following integration, the provirus is maintained as a stable genetic element in the infected cell and its progeny. Most or possibly all regions of the host genome are accessible to retroviral integration (Withers-Ward et al., Genes Dev. **8**:1473-1487 (1994)), and the above properties make retroviruses invaluable as both potent mutagens and chromosomal markers.

The MAGEKO process employs one or more retroviral vectors as mutagens. The principal vector is preferably based on the Moloney murine leukemia virus (MoMLV) (Varmus and Brown, supra). Secondary vectors are of different retroviral origin and include, for example, lentiviral (Varmus and Brown, supra) or avian leukosis-sarcoma virus (ALV) (Varmus and Brown, supra) based vectors. Different retroviral backbones are utilized in the MAGEKO technique to increase the number of genes that are affected by insertional mutagenesis, on the theory that different retroviruses may have different genomic targeting preferences. Furthermore, in the case of the lentiviral vectors, it is known that this retrovirus is capable of transducing nondividing cells (Naldini et al., Science **272**:263-267 (1996)), thus allowing for earlier detection of infected cells. Integration of vectors involving MoMLV depends on mitosis (Roe et al., EMBO J. **12**:2099-2108 (1993)).

Each vector used in the mutagenesis procedure is quite similar, differing significantly only in the retroviral backbone sequence. Otherwise, the vectors carry in common several unique features essential to the subsequent functional characterization of the inactivated genes of interest. In particular, as discussed in more detail below, each of the vectors is highly mutagenic, each allows rapid identification of infected cells, and